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ENHANCED SDF-1 AND RANTES ATTRACT THE LOCALLY INJECTED BONE MARROW STROMAL CELLS TO REMEDY DENTAL BIOMECHANICALLY INDUCED TEMPOROMANDIBULAR JOINT OSTEOARTHRITIC LESIONS

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Purpose: The self-repairing capability of the articular cartilage is limited so that cell-based therapy holds promises on osteoarthritis (OA). Currently we adopted the mice with temporomandibular joints (TMJs) OA lesions induced by applying a dental biomechanical stimulation termed unilateral anterior crossbite (UAC) developed in our lab. TMJ local injection of GFP labelld bone marrow stromal cells (GFP-BMSCs) was performed on the UAC mice. The purpose was to investigate the remedy effect of the locally injected BMSCs on OA lesions and the underlying mechanisms.
Methods: UAC was applied to the 6-weeks old C57BL/6J mice. The GFP-BMSCs were injected to the right side TMJ area 3-weeks after UAC operation. Histological and three-dimensional microcomputed tomography analyses, immunohistochemical (IHC) and immunofluorescent (IF) staining methods were used to evaluate the OA lesions and the remedy effects. TMJ organ and GFP-BMSCs co-culture assay was adoted to detect the migration and differentiation activity of the GFP-BMSCs. Bioluminescence imaging technique was used for tracking the injected GFP-BMSCs. TMJ local injection of AMD3100 or/and BX471, the antagonist of SDF-1/CXCL12 and ROUNTS/CCR4 signalling was performed for detection of the role of these two signals on the GFP-BMSCs implantation and their remedy effect on the OA cartilage.
Results: 1. Weekly local delivery of the GFP-BMSCs successfully rescued the OA lesions at 4 and 8 weeks after injection. The parameters as revealed by histological and three-dimensional microcomputed tomography analyses reached the control levels at 12 weeks. IHC and IF staining confirmed that the GFP positive cells implanted in the UAC TMJ cartilage lesion area became Col-II positive cells but rarely expressed Col-I or OCN, implying they are chondrocyte differentiated without further differentiation into osteoblasts or fibroblasts. 2. The in vitro data indicated that when a condyle from either UAC or control group was implanted in the upper chamber and the GFP-BMSCs in the lower chamber of the transwell system, the condyle from both groups induced chondrogenesis of the co-cultured GFP-BMSCs revealed by expression of proteoglycan or Col-II. However, when a condyle from either UAC or control group was co-cultured directly with the GFP-BMSCs in a plate, more GFP-positive cells were found in the UAC TMJs condylar cartilage but less in controls. That means UAC TMJ has a higher attractive capacity to recruit BMSCs to the cartilage. This result was confirmed by the Bioluminescence test. After injecting the luciferase-labeled BMSCs into the UAC TMJ regions 3-weeks post-operation, the luminescent intensity was found gradually increased, reached the peaks at d7, and then declined until d28, the end of the experimental period. 3. Real-time PCR and IHC data revealed that in the TMJ cartilage there was an increased expression of SDF-1 and RANTES versus the age-matched control groups. When a TMJ condyle was placed in the lower chamber and GFP-BMSCs in the upper chamber of the transwell system, more BMSCs migrated from the upper chamber towards the lower chamber when co-cultured with a UAC condyle versus a control condyle. This higher BMSCs migration capacity was attenuated by CXCR4 antagonist AMD3100 or by CCR1 antagonist BX471 in a dose-dependent manner, and was almost completely attenuated when both CXCR4 and CCR1 inhibitors (20 μM AMD3100 and 20 μM BX471) were applied together. 4. Injection of AMD3100 and BX471 combined with administration of BMSCs to the TMJ local area impaired the GFP-BMSCs implantation and their remedy effect on cartilage repair.
Conclusions: SDF-1/CXCR4 and RANTES/CCR1 mediate recruitment of the exougenous BMSCs to the OA-lesion sites where the recruited BMSCs differentiate into chondrocytes to remedy the OA-like lesions.

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THE ELASTIC NETWORK IN GROWING CARTILAGE

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Purpose: The aim of this study is to investigate the organization of elastic network in developing cartilage, ultimately to understand the contribution of the elastic network in cartilage biomechanical function and its role in aging and osteoarthritis development.
Methods: Fresh metacarpal-phalangeal joints from 7-day-old calves were collected from a local abattoir. Plugs of articular cartilage from metacarpal joints encompassing the surface layer to the subchondral bone were dissected and immediately snap frozen and stored at -80 °C till used. Cryostat sections, 20 μm thickness, were cut and mounted on super-frosted slides. Dual immunostaining of fibrillin-1 with either elastin or fibrillin-2 was carried out as previously described (1). All sections were examined using a conventional fluorescence microscope.
Results: As seen in Figure 1: An elastin network was only observed in the superficial layer of the articular cartilage; in the deeper zones its presence was sparse. By contrast, an extensive and well-organised network of fibrillin-1 and fibrillin-2 was distributed throughout the young cartilage in the entire region lying between surface of the layer of the growing cartilage and the second ossification center. Fibrillin-1 and fibrillin-2 co-localised except in the region of the secondary growth plate. In the region of the articular cartilage, the fibrillins appeared to lie parallel to the cartilage surface, but in the cartilage depth, the colocalised fibrillins appeared to encircle groups of cells, forming compartments.
Conclusions: Here, using dual immunostaining of elastin and fibrillins we describe our finding of an extensive network of fibrillins in the young growing cartilage. Unlike in most tissues, the fibrillin network was not co-localised with elastin except at the articular cartilage surface. Away from the cartilage surface, the fibrillin network appears to form compartments enclosing groups of cells. These compartments are similar in appearance to those seen in elastic cartilages of the ear although, unlike in ear cartilage, these appeared to consist only of fibrillin-containing microfibrils with no elastin fibres apparent. Fibrillins persist into adult cartilage but their organization is very different; there they form a fine filamentous network. Their role in growing and adult articular cartilage is unknown. The function of fibrillins is biomechanical in some tissues; fibrillins are also involved in regulation of TGF beta activity. Whatever their role, the density and detailed organization of these microfibrils in growing and adult cartilage indicate their role is of some importance and requires further investigation, particularly in regard to cartilage repair and cartilage pathology.
Reference:
1. Yu J, et al (2007) Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. J Anat 210, 460-471.